EXHIBIT 1

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Homopolymeric Tract Heteroplasmy in mtDNA from Tissues and Single Oocytes: Support for a Genetic Bottleneck

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Summary

While mtDNA polymorphisms at single base positions are common, the overwhelming majority of the mitochondrial genomes within a single individual are usually identical. When there is a point-mutation difference between a mother and her offspring, there may be a complete switching of mtDNA type within a single generation. It is generally assumed that there is a genetic bottleneck whereby a single or small number of founder mtDNA(s) populate the organism, but it is not known at which stages the restriction/amplification of mtDNA subtype(s) occur, and this uncertainty impedes antenatal diagnosis for mtDNA disorders. Length polymorphisms in homopolymeric tracts have been demonstrated in the large noncoding region of mtDNA. We have developed a new method, T-PCR (trimmed PCR), to quantitate heteroplasmy for two of these tracts (D310 and D16189). D310 variation is sufficient to indicate clonal origins of tissues and single oocytes. Tissues from normal individuals often possessed more than one length variant (heteroplasmy). However, there was no difference in the pattern of the length variants between somatic tissues in any control individual when bulk samples were taken. Oocytes from normal women undergoing in vitro fertilization were frequently heteroplasmic for length variants, and in two cases the modal length of the D310 tract differed in individual oocytes from the same woman. These data suggest that a restriction/amplification event, which we attribute to clonal expansion of founder mtDNA(s), has occurred by the time oocytes are mature, although further segregation may occur at a later stage. In contrast to controls, the length distribution of the D310 tract varied between tissues in a patient with heteroplasmic mtDNA rearrangements, suggesting that these mutants influence segregation. These findings have important implications for the genetic counselling of patients with pathogenic mtDNA mutations.

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Introduction

Mutations in human mtDNA accumulate ~10 times faster than in nuclear DNA so that mtDNA polymorphisms are common. There are thousands of copies of mtDNA in a single cell, and yet most of the mitochondrial genomes in a single human control are identical (homoplasmy) (Monnat and Loeb 1985). In contrast, heteroplasmy (two or more populations of mtDNA in a single individual) is common in patients with mtDNA diseases. The reason for this is unknown, but it may be that in some cases homoplasmy for mutant mtDNAs is lethal.

Despite the fact that there are thousands of mtDNAs in a cell, when there is a neutral point mutation difference between a mother and her offspring, there may be complete switching of mtDNA genotype in a single generation, as has been demonstrated in Holstein cows (Koehler et al. 1991). If this switching were caused by random segregation or drift, heteroplasmy for polymorphic point mutations should be relatively common. In practice, it has been reported only rarely. Alternatively, the state of homoplasmy may be maintained by a restriction/amplification event, or "bottleneck," whereby a small number of mtDNA molecules ultimately populate the organism. By analyzing instances where segregation is not complete, investigators have estimated the number of mtDNAs that may be founders of the adult genotype. Estimates range from 1–6 (Hauswirth and Laipis 1985) and 20–100 in cows (Ashley et al. 1989) to 370–740 segregating units in *Drosophila* (Solignac et al. 1987). The segregating unit could represent the mtDNA complement of a single mitochondrion or subgroup of mitochondria in humans. It is not known at which stage this restriction/amplification of one mtDNA subtype might occur, nor is its molecular basis known. However, there is a 50-fold increase in the number of mtDNAs per cell, from 4,000 to 200,000, during oogenesis. Daughter cells could be clonal, with respect to mtDNA, if they originate from regions of cytoplasm within the oocyte where clones derived from founder mtDNAs remain in clusters (Hauswirth and Laipis 1985).

Length polymorphisms have been demonstrated in homopolymeric tracts within the large noncoding region of mtDNA (Hauswirth and Clayton 1985; Bendall and Sykes 1995). Heteroplasmy of this length variation in

homopolymeric tracts has been recognized because of blurring of bands on a sequencing gel after the tract. That this was not a sequencing artifact was demonstrated by cloning and sequencing of individual clones, at which point the sequence becomes readable (Bendall and Sykes 1995; Marchington et al. 1996). In order to identify heteroplasmic variants that might be used as markers for different mtDNA clones within the germline and somatic cells of normal individuals, we investigated the variation in two of these homopolymeric tracts. Minisatellite repeats with extremely high mutation rates have been used to investigate nuclear variation in germ-line and somatic cells (Monckton et al. 1994), while point mutations, which evolve more slowly, are suitable for more deeply rooted relationships. By analogy, we required a mtDNA polymorphism with a high rate of variation for these studies. We have developed a new method, T-PCR (trimmed PCR) to quantitate heteroplasmy for two mtDNA homopolymeric tracts (D310 and D16189) in tissues and oocytes (table 1). We found that D310 was sufficiently stable for length variants to cosegregate with clonal mtDNA populations but that D16189 was not suitable. We have used an analysis of length variation of D310 to investigate the segregation of mtDNA molecules within single oocytes of humans and mice.

Subjects and Methods

Patient Material

Ethical approval was obtained from the Central Oxford Research Ethics Committee. Oocytes that had failed to fertilize 2 d after insemination in vitro were donated for research by five women undergoing in vitro fertilization (IVF). Three to eight oocytes from each woman were available and used in this study. Oocytes were incubated in individual petri dishes. Oocytes were removed from the dishes and washed in sterile PBS. Adherent sperm and the zona pellucida were removed by brief exposure to PBS adjusted to pH 2.6, a modification of the acidic Tyrode solution method. The oocytes were placed in fresh PBS and checked for the absence of sperm. Thus, oocyte mtDNA was in a vast excess over any residual traces of paternal mtDNA derived from any adherent sperm.

Two to five postmortem tissues samples were obtained from five subjects who died from nonneurological causes (controls). Patient 1 had Kearns-Sayre syndrome (KSS) and rearranged mtDNA, comprising duplications, deletions, and deletion dimers, in addition to normal mtDNA as described by Poulton et al. (1995). Nine different tissues were available. Length variation in D16189 was previously reported in patient 2 (Marchington et al. 1996), who had a T:A→G:C transition at bp 3243, which is a pathogenic mutation associated with MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes).

Mice

Oocytes were dissected from the ovaries of a C57BL/6 mouse. The ovaries were washed and placed in sterile PBS. The tissue was dissected in PBS under a dissecting microscope and the oocytes released were removed and placed in fresh PBS. The oocytes were examined by light microscopy, and any that were damaged or had adherent cells were discarded. Nineteen intact oocytes were obtained.

Table 1

T-PCR of the Variable Tracts

	Tract		
Tract Name	D16189	D310	Mouse D310 Homologue
Wild-type sequence	CCCCCTCCCC	CCCCCCCTCCCCC	CCCCCCACCCCC
Normal situation	Homoplasmic	Heteroplasmic	Heteroplasmic
Forward primer (bp)	16161–16183	266–285	16068–16087
Forward primer sequence	TAAAAACCCAATCCACATCAAA	TTCCACACAGACATCATAAC	CCAAATTTTAACTCTCCAAA
Reverse primer (bp)	325–344	580–599	10–31
Reverse primer sequence (biotinylated)	AGATGTGTTTAAGTGCTGT	TTGAGGAGGTAAGCTACATA	GTGCTTTGCTTTGTTATTAAGC
Polymorphism giving rise to length variation	15% Caucasians	Not applicable	Not applicable
Restriction enzyme	*Rsa*I	*Hae*III	*Dde*I
Recognition/cutting site	GT→AC 16209	GG→CC 323	C→TAAG 16127
Size of PCR product (bp)	752	333	258
Size of fragment released (bp)	48+/− tract-length variation	57+/− tract-length variation	59+/− tract-length variation

T-PCR

We investigated two homopolymeric tracts within the large noncoding region of mtDNA near bp 16189 and 310 (henceforth D16189 and the D310 tract, respectively; see table 1). In most individuals, the D16189 tract consists of a run of 10 C:Gs, with a T:A at bp 16189. However, in ~15% of Caucasians, there is a T:A→C:G mutation at bp 16189. The homopolymeric tract resulting from this mutation may vary from 8 to 14 bp in length. The D310 tract consists of a run of 12–18 C:Gs, with a T:A near the middle at bp 310. To quantitate the proportions of each length variant, we have developed a PCR-based method. Short PCR products of ~40–50 bp were end-labeled with ^{32}P and separated on sequencing gels, and the distribution of the length variants was quantitated by phosphorimager analysis and autoradiography. This simple procedure may give rise to artifactual length variants because *Taq* polymerase can introduce a 1-bp overhang at the 3′ end in PCR products, and primers are usually contaminated with low levels of incomplete oligonucleotides. Furthermore, single-stranded DNA and its complementary strand may migrate to different positions on a denaturing gel. Primer dimers also run at 40–50 bp. We developed T-PCR, to avoid these confounding problems, and figure 1*A* shows a scheme for T-PCR of the D310 tract. PCR products of several hundred base pairs, encompassing the tract of interest, were generated (see table 1). A 5′-primer (PAGE purified to a single length for incorporation into the final product), adjacent to the tract, was used with a biotinylated antisense primer (which blocks end-labeling of that strand). PCR products were immobilized by binding to streptavidin-coated beads (Dynal). The immobilized products were washed, end-labeled (on the nonbiotinylated strand only) with γ-^{32}P dATP by polynucleotide kinase, washed, and incubated with a restriction enzyme that cuts just downstream of the homopolymeric tract of interest. The size of the labeled product cleaved from the beads reflects the length of the homopolymeric tract and is used to quantitate length variants. The 3′ end with heterogeneous overhang and possible contaminants, such as primer dimers and products that result from false priming, remains attached to the beads. The short labeled products were free from unincorporated label and were heated at 75°C for 2 min with a denaturing buffer before being run on an 8% sequencing gel at 75 W for 2–3 h. Gels were dried under vacuum and exposed to X-ray film or phosphorimager plates.

In order to ensure that any apparent heteroplasmic length variation was not a PCR artifact, PCR products containing the D310 tract were cloned into M13 (the host was DH5α, which is recA$^-$). Clones were reamplified by PCR, and these PCR products were cloned. Fifty of these clones were sequenced, and in all cases the track length was identical. Cloned DNA was amplified by T-PCR, and at no time was more than a single length obtained (results not shown).

Standard PCR conditions were used (Marchington et al. 1996) for 40 cycles, in all cases, and the starting quantities of template were ~10^4 and 10^8 copies of mtDNA per reaction for eggs and tissues, respectively. We demonstrated that the distribution of length variants was not influenced by low copy number (which might result in "allelic dropout") by diluting tissue samples down to a level below the limit of detection of any product ($<10^2$). We confirmed that T-PCR was quantitative by mixing, in various proportions, DNA samples (from tissues or cloned DNA as above) that were known by sequence analysis to have D310 tracts of different length and analyzing them by T-PCR (figs. 1*B* and 1*C*). Figure 1*B* shows autoradiographs obtained by mixing DNA from two different individuals' muscle tissue. Figure 1*C* demonstrates that, after quantitation by a phosphorimager, the proportion of the signal attributable to each sample varied with their proportion in the sample.

Results

The variation rate of the D310 and D16189 tracts were compared in cybrid lines (courtesy of Dr. I. J. Holt) derived from a patient with both the 3243 mutation associated with MELAS and the 16189 T:A→C:G polymorphism (Marchington et al. 1996). The cybrids were derived by fusing cytoplasts from patient 2's myoblasts with a mtDNA-free cell line. Figure 2 illustrates length variation in D310 and D16189 tracts in muscle tissue and muscle cell cybrids from patient 2 containing different proportions of 3243 G:C mutation In the D310 tract, the 0% mutant sample (lane 2) clearly segregates with a different major length variant (length variant 3) relative to the samples containing high levels of the 3243 G:C mutation (lanes 1, 3, and 4), which segregate with length variant 2. However, D16189 tract variants displayed the same distribution, irrespective of the proportions of 3243 G:C mutation, demonstrating that the same proportion of 3243 mutation was present in each length variant. Thus, in this culture system, specific D310 variants cosegregated with the 3243 mutant, but D16189 variants did not.

Control individuals were investigated for heteroplasmic length variation in the D310 tract in 2–5 different tissues. Figure 3 shows that the length of the D310 tract can vary between individuals. Subjects 2, 3, and 5 were homoplasmic, with a single length variant in the D310 tract. In subjects 1 and 4, there were low levels of a shorter-length variant. In subject 1, this varied from 1% to 4% and in subject 4 from 4% to 5%. Thus, although there may be some degree of heteroplasmy within the five normal individuals, a single modal length accounted for $\geq$95% of the length variants within an individual.

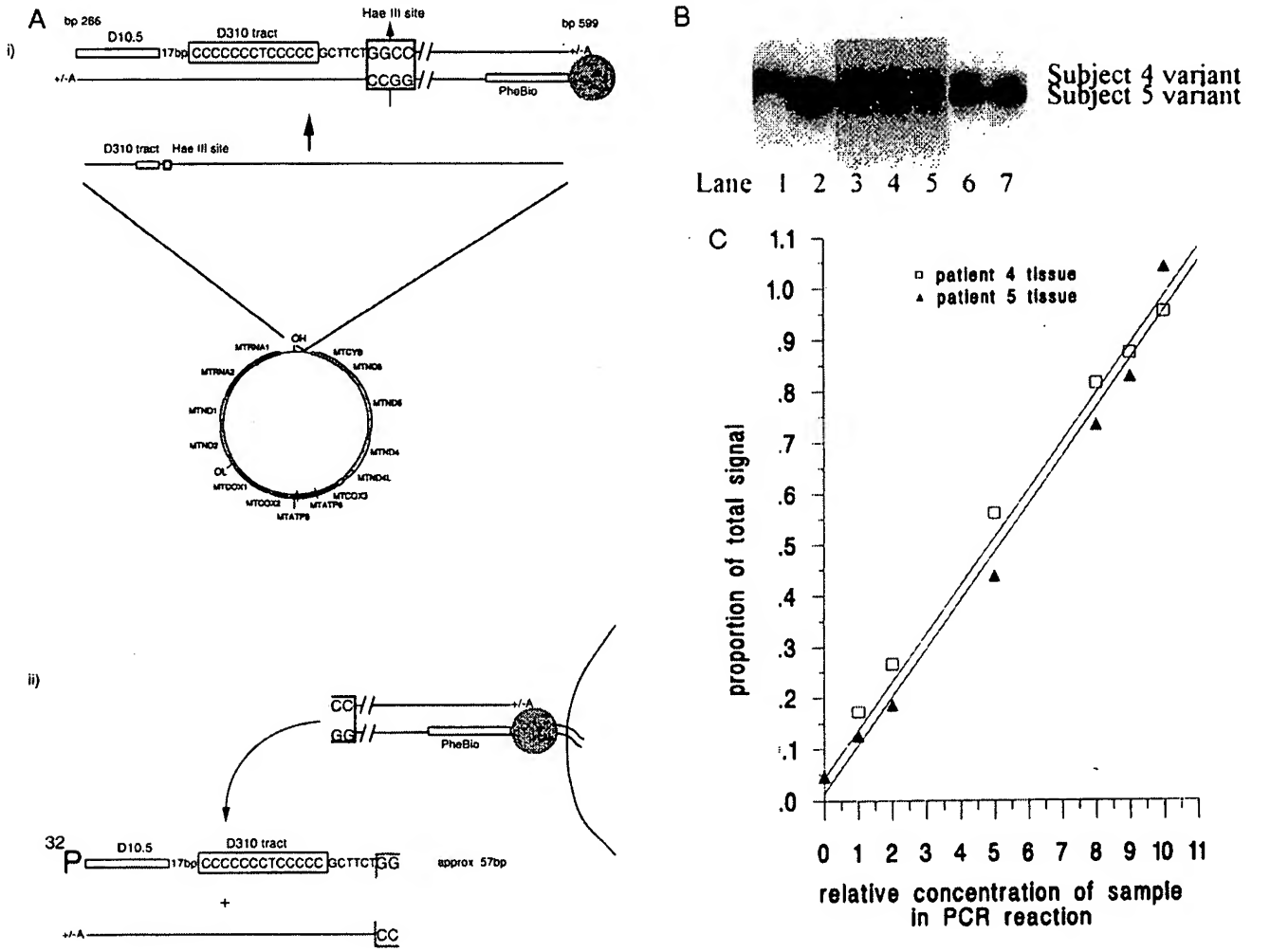
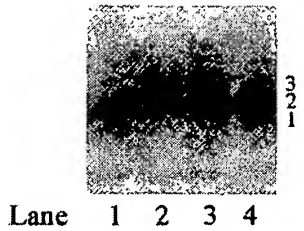
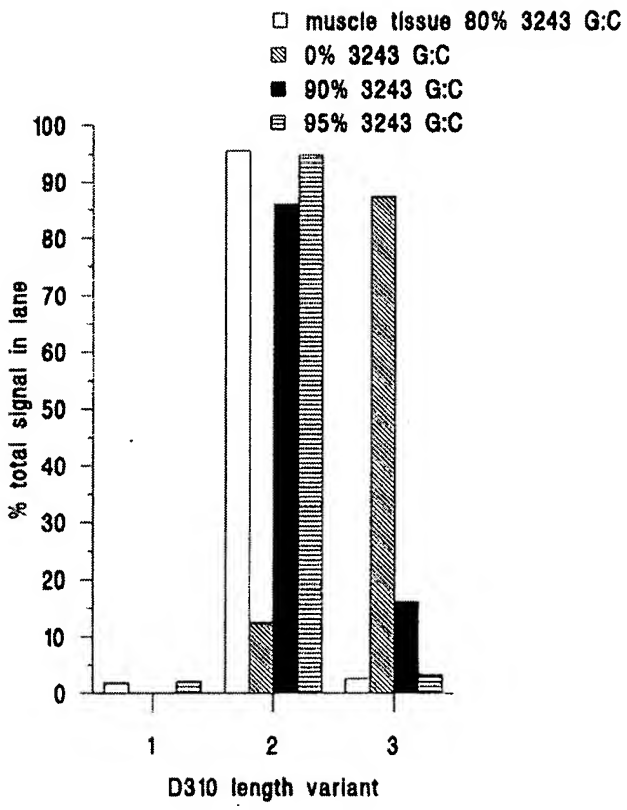


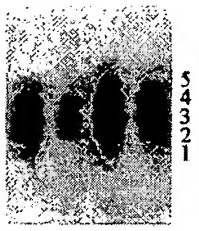
Figure 1 D310 tract T-PCR. *A*, Scheme of T-PCR for the D310 tract. (i) The 5′ primer (D10.5) was selected to be slightly upstream of the D310 tract, and a *Hae*III site was identified just downstream of the tract. The 3′ biotinylated primer (PheBio) was positioned several hundred base pairs downstream of the tract to provide a long "handle" and to ensure separation of products after digestion with the restriction enzyme. (ii) The PCR products were immobilized on streptavidin-coated beads, and the strand containing the PAGE-purified D10.5 primer was end-labeled with ^{32}P (the biotin blocks labeling of the other strand). Incubation of the beads with the restriction enzyme *Hae*III releases a labeled product of ~57 bp (whose exact size reflects the length of the homopolymeric tract and which was used to quantitate length variation therein). The other product of the digestion (276 bp) remains attached to the beads. *B*, Muscle DNA from two subjects (4 and 5) with different length D310 tracts, which were mixed in various proportions (v/v) and samples subjected to T-PCR as described in Subjects and Methods. Mixings are as a ratio of patient 4:patient 5: Lane 1, 10:0; Lane 2, 0:10; Lane 3, 9:1; Lane 4, 8:2; Lane 5, 5:5; Lane 6, 2:8; and Lane 7, 1:9. *C*, T-PCR products, which were quantitated by phosphorimager. For each band, the proportion of total signal in the lane was calculated and plotted as a function of the concentration of the sample in the PCR reaction. This demonstrates that T-PCR can be used to quantitate accurately length variants in the D310 tract. Muscle from subject 4 was heteroplasmic for length variation and contained 4% of the shorter variant found in subject 5; this is reflected in the displacement of the assay point with 0% subject 5 DNA by this amount.

Single oocytes were examined for evidence of mtDNA heteroplasmy. Figure 4 illustrates D310 tract variation in single oocytes from a single mouse ovary and in single human oocytes from patients attending the Oxford IVF Unit. Almost all oocytes were heteroplasmic. Figure 4*A* shows that, in the mouse, ~25% of oocytes differed from the mean pattern, and there were two cases, lanes 1 and 8, where the major length variant was also different. In human oocytes (fig. 4*B*), the tract variation pattern was the same in all oocytes from each of three of five donors (subjects 7–9 in fig. 4*B*). In the other two subjects (6 and 10) there are at least two different major length variants.

Figure 5 shows that there was a greater degree of heteroplasmy and variation of the D310 tract between tissues in a patient with mitochondrial disease than in controls, and this was confirmed by phosphorimager analysis. There were two length variants in each tissue, but the proportions varied. The extremes of variation were between spleen (lane 2) and liver (lane 7). In spleen,







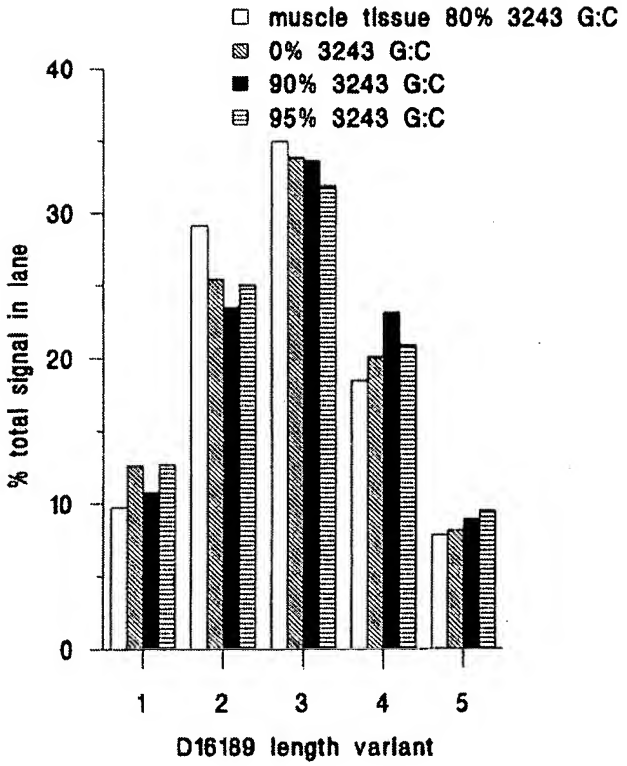


Figure 2 Comparison of length variation in different homopolymeric tracts and cloned muscle cell hybrids from patient 2, who is heteroplasmic for a pathological point mutation at bp 3243. Length variation was assessed in D310 (*upper panel*) and D16189 (*lower panel*) tracts by T-PCR. Lane 1, muscle (80% 3243 G:C). Lanes 2–4, muscle-cell hybrids containing 0%, 90%, and 95% 3243 G:C, respectively. The graphs show phosphorimager analysis of the T-PCR products; the length variants are numbered as on the autoradiographs. In the D310 tract, length variant 2 tends to segregate with the 3243 mutation, while length variant 3 tends to segregate with wild type. However, in the D16189 tract, length variants display the same distribution, irrespective of the proportions of 3243 G:C mutant.

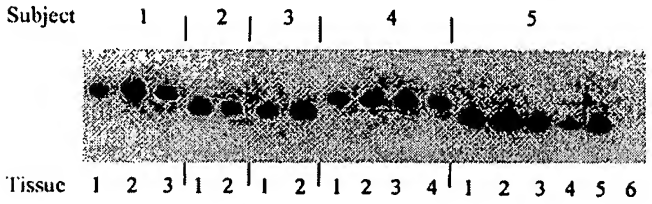


Figure 3 D310 length variation in tissues from controls. The autoradiographs show T-PCR of DNA from tissues of five subjects as described in Subjects and Methods. The tissues were (from *left* to *right*) subject 1, (1) muscle, (2) heart, and (3) liver; subject 2, (1) heart and (2) liver; subject 3, (1) muscle and (2) heart; subject 4, (1) muscle, (2) heart, (3) liver, and (4) kidney; subject 5, (1) heart, (2) liver, (3) kidney, (4) brain, and (5) pectoralis. Lane 6, subject 5, a water control. This demonstrates that bulk tissue samples may be heteroplasmic but that the modal length is identical in all tissues studied in an individual.

5% of the signal was found in the lower band, whereas in liver the value was 40%.

Discussion

We have used length variation in homopolymeric tracts around bp 310 and 16189 of mtDNA to investigate heteroplasmy in normal controls and patients with mitochondrial disease. We suggest that it is legitimate to use D310 but not D16189 as a marker of clonal origin of mtDNA. Figure 2 shows that, in contrast with the D310 tract, no particular D16189 variants cosegregated with the 3243 G:C mtDNAs in the MELAS patient. This suggests that, in cell culture, length variants were generated more rapidly in D16189 than in the D310 tract. Therefore, rapid generation of D16189 variants would conceal segregation of mtDNA clones. It has been shown that an identical distribution of D16189 variants can be passed down a pedigree (Bendall and Sykes 1995; Marchington et al. 1996), and this has been used as evidence against a narrow mtDNA bottleneck. Conversely, a bottleneck could be concealed by length variation generated during oogenesis and/or embryogenesis in these maternal lineages, as occurred in our MELAS patient cell lines. Therefore, we suggest that the D310, but not the D16189, tract may be a useful marker of mtDNA clonality. In both cases, length variation is presumably caused by replication slippage (Hauswirth et al. 1984), but it is not clear why there is a difference in the rate of generation of length variants between the two tracts. While the presence of a T:A base pair interrupting the C:G tract appears to ensure stability in the wild-type D16189 tract, this interruption is not sufficient to stabilize the D310 tract. Once the T:A base pair in the D16189 tract is lost, this tract may become markedly less stable than the D310 tract. However, the presence of this T:A base pair in the D16189 tract cannot be the only determinant of stability, because a continuous C:G tract is not always associated with length variation (Marchington et al. 1996).

We have confirmed that the D310 tract may vary in modal length between individuals (Horai and Hayasaka 1990). Other authors have shown that heteroplasmic

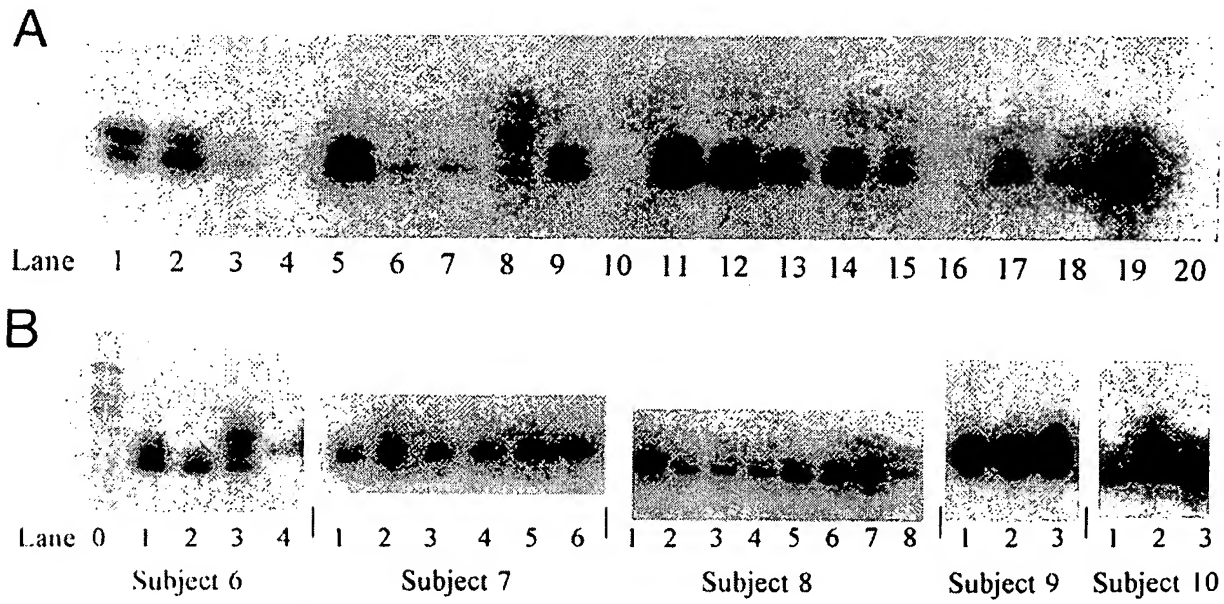


Figure 4 D310 tract variation in single oocytes. *A*, Mouse equivalent of the D310 tract, investigated by T-PCR in single oocytes dissected from a mouse (C57BL/6) ovary, lanes 1–19. Lane 20 is a water control. *B*, Autoradiographs, which show D310 tract variation, assessed by T-PCR, in single oocytes from the Oxford IVF Unit (subjects 6–10). Lane 0 in the subject 6 panel is a 1-bp sequencing ladder. This demonstrates that the modal length of the D310 tract may vary between oocytes from a single control.

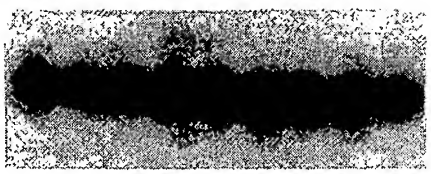


Figure 5 D310 length variation in a patient with KSS. The autoradiographs show T-PCR of DNA from tissues of patient 1. The tissues were (1) pituitary, (2) spleen, (3) muscle, (4) heart, (5) brain, (6) pancreas, (7) liver, (8) ovary, and (9) pectoralis. This demonstrates that the mean length of the D310 tract may vary between tissues in this patient.

substitutions and single-base-pair insertion/deletion mutations occur at a high frequency in the D310 tract (Jazin et al., in press). We have demonstrated that normal individuals may be heteroplasmic for D310 length variants. While heteroplasmy is frequently reported in mitochondrial disease, presumably because homoplasmy for many of the underlying pathogenic mutations would be lethal in many instances, heteroplasmy for mitochondrial polymorphisms has been reported only occasionally (Howell et al. 1992; Jazin et al., in press).

In normal individuals, there may be a degree of heteroplasmy in the D310 tract length in the tissues investigated. Moreover, the distribution of length variants of this tract appears to be identical among different tissues within each individual (fig. 3). This suggests that the mtDNA in each bulk tissue, within a normal individual, arises from the same founder mtDNA(s). The small number of additional length variants, which may be generated by replication slippage during expansion of the mtDNA population, may be masked by the excess of the modal length variant.

We found more length variation in single oocytes from humans and mice than in bulk samples from tissues. Although the human oocytes had been in the presence of sperm, it is highly unlikely that they could have been contaminated by significant quantities of sperm mtDNA. A single sperm contains ~50 mtDNAs (Hecht et al. 1984). Any sperm mtDNA that had penetrated the oocyte would be diluted by a factor of 2,000. It is conceivable that some sperm mtDNA might have been solubilized following removal of the zona in acid PBS and been transferred with a small volume of PBS in subsequent washes. If so, it would have been diluted in excess of 2,500-fold in the PBS and a further 2,000-fold by the maternal mtDNA. It is highly unlikely that contamination on this scale could result in a shift in the modal length of the PCR product. In addition, similar results were obtained with mouse oocytes that had never been exposed to sperm. Therefore, any mtDNA from nonoocyte ovarian tissue would have been diluted out ≥10,000-fold as with the human oocytes–washing/transfer procedure, and tract variants most likely arose from the oocyte mtDNA.

The presence of different modal lengths of the D310 tract in individual oocytes from "normal" women suggests that a restriction/amplification event must have occurred between conception and maturation of oocytes. For instance, in subject 10 in figure 4*B*, the difference in modal length between the oocyte in lane 1 (or lane 3) as opposed to the oocyte in lane 2 suggests expansion of different founder mtDNAs in the two oocytes. This difference could have arisen by clonal expansion of a single or a few mtDNAs within each oocyte, by segregation with drift, or by a combination of both. We suggest that clonal expansion of founder mtDNAs may make the major contribution to this difference, since there is a 50-fold increase in the number of mtDNAs during oogenesis in cows, from ~4,000 in oogonia to ~200,000 in mature oocytes, coincident with a reduction in the number of mtDNAs per mitochondrion (Hauswirth and Laipis 1985). The smaller the number of segregating units for mtDNA in the germ line, the more likely it is that mtDNA in the progeny will be clonal, provided that all the mtDNA molecules in one segregating unit are identical. However, the high mutation rate of mtDNA, particularly in the large noncoding region that includes the D310 tract, may allow a degree of length variation in the tract by the time the organism is mature. As with the bulk tissue samples, any minority length variants may be barely detectable, unless cells that are clonal with respect to mtDNA, such as in individual oocytes, are selected. The number of founder mtDNAs cannot be estimated where multiple D310 length variants were detectable because new lengths may be generated during the course of clonal proliferation. However, a difference in the modal tract length in two samples from the same individual is highly suggestive of different founder mtDNAs. The presence of different modal lengths of the D310 tract in individual oocytes in a proportion of "normal" women is the first direct evidence suggesting that restriction/amplification of founder mtDNA(s) has occurred by the time normal human oocytes are mature. Such clonal expansion could underlie the rapid switching of apparently neutral polymorphisms between generations. Information about the timing of this restriction/amplification event is an essential prerequisite if prenatal diagnosis of mtDNA disorders is to become feasible. Provided that the major component of this rapid switching occurs in the female germ line, quantitation of the level of mutant mtDNA in chorionic villus may be a good reflection of the level in the whole embryo.

Because the oocyte donors were attending an infertility clinic, it is not certain that these oocytes would have been viable and hence represent the normal situation

accurately. However, as before, similar results were obtained on oocytes from a normal virgin mouse (fig. 4*A*). The oocytes have been through some sort of restriction/amplification event, whether or not viability is low. This is also consistent with unpublished findings in a mouse model of heteroplasmy: the proportions of two populations of mtDNAs in mature oocytes reflected those in the newborn offspring, suggesting that a restriction/amplification event or "bottleneck" precedes the final maturation of oocytes (Jenuth et al. 1996). Rapid segregation could contribute to this switching of mtDNA populations in pathogenic mtDNA mutations, which may occur in vitro (Yoneda et al. 1992; Dunbar et al. 1995). We investigated two patients with pathogenic mtDNA mutations, both of whom were heteroplasmic for wild-type and mutant mtDNA. Figure 5 shows a greater degree of length variation between tissues in a patient with a heteroplasmic rearrangement of mtDNA (Poulton et al. 1995) than in controls (fig. 3). This may indicate that additional factors (such as the effect of impaired mitochondrial function on cell growth or faster replication of mutant than wild-type mtDNAs in certain tissues) may influence transmission of mtDNA in disease. Segregation of length variants in this case may depend on cosegregation with members of the different populations of mutant mtDNA. Furthermore, it is likely that further changes in the proportion of mtDNA mutant occur during subsequent clonal expansion and after birth (Poulton and Morten 1993; Matthews et al. 1994). This does not conflict with earlier views on the bottleneck. Segregation studies in mtDNA disease have suggested that several mtDNAs, rather than a single mtDNA, generally populate the progeny of affected females. The segregating unit may be a mitochondrion that contains several mtDNAs and hence is potentially heteroplasmic. Without knowing whether individual mitochondria can be heteroplasmic, attempts to calculate the number of segregating units may be flawed. However, rapid switching between different mtDNA types occasionally occurs, suggesting that a segregating unit may be homoplasmic for wild type. Attempts to calculate the number of segregating units in normal individuals are few. Howell et al. (1992) found heteroplasmy at a polymorphic site (bp 14,560) in a family with Leber hereditary optic neuropathy due to a pathogenic mutation at bp 3460 (which was homoplasmic). At bp 14,560, there was a silent G:C→A:T transition at the third base position in a codon specifying valine. The proportion of mutant ranged from 22% to 66% in the progeny. This failure of wild-type and mutant mtDNA to segregate to homoplasmy suggests again that mtDNAs that are wild type and mutant at bp 14,560 could be grouped together in segregating units. It could perhaps be maintained by large numbers of mtDNAs per mitochondrion or conceivably by aggregation of mtDNAs in multimeric forms such as catenates. For instance, mutant resolvases in yeast may result in biased transmission by aggregation of unresolved multimeric mtDNAs into large segregating units (Lockson et al. 1995).

Conclusion

These data support the concept of a mtDNA bottleneck whereby a few founder molecules populate the organism by demonstrating more mtDNA variation between oocytes than between tissues within single individuals. We suggest that a bottleneck occurs in oogenesis before the formation of mature oocytes. If this is the major determinant of mtDNA segregation between generations, antenatal diagnosis for mtDNA diseases may become feasible. However, it is likely there may be further segregation during development (Matthews et al. 1994).

Acknowledgments

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